Development of hemocyte primary culture and identification of immune cytokines in locusts

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1. INTRODUCTION

The entomopathogen-based locust biocontrol may present a sustainable solution to the social and environmental limitations posed by the current chemical control. However, for optimal use of the biocontrol, the locust immunity should be effectively compromised, directing the need for comprehensive understanding of the locust immune system.

The locusts seem to depend largely on a cell-mediated immunity due to a rare presence of AMP like humoral factors in their immune system. However, the cell-mediated immune system is poorly characterized and there is no established *in vitro* method to study the locust cell immune system. Hence, we developed an *in vitro* method for the locust cellular immunity studies and attempted to identify cytokines mediating the immune system, hemocyte activation factors.

2. MATERIALS AND METHODS

L. migratoria (Lm) hemocyte *in vitro* culture

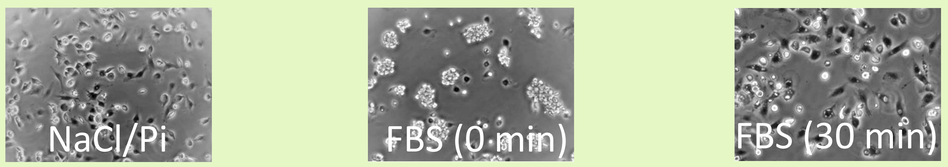
- **Requirements**: Grace basal medium, 380mOsm, pH 6, 2-Mercaptoethanol, Phenylthiourea**,** Fetal bovine serum (FBS), 28°C, medium change 3x per week
- ***In vitro* validation tests**: Cell survival, phagocytosis, proliferation

Screening for immune-associated cytokines
(*Locusta migratoria* and *Schistocerca gregaria*)

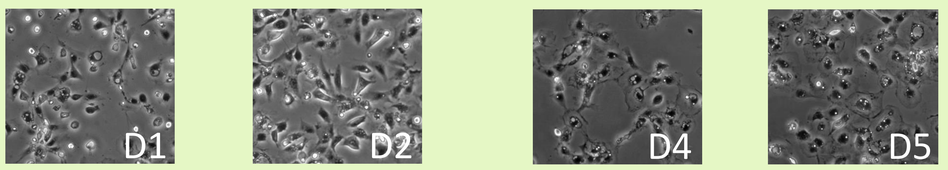
Database screening (NCBI, EST, Max Plank) → Peptide synthesis - Cloning & expression - Synthetic production → *In vitro* test (hemocyte activation)

3. Lm HEMOCYTE CELL CULTURE

Effect of FBS addition in Lm hemocyte culture

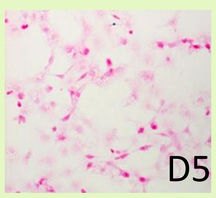


Lm hemocytes form monolayer *in vitro*

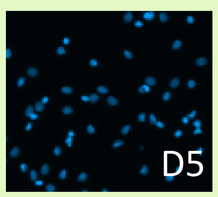


Validation of Lm hemocyte cell culture

Phagocytosis *in vitro*
C. albicans-FITC (Green)



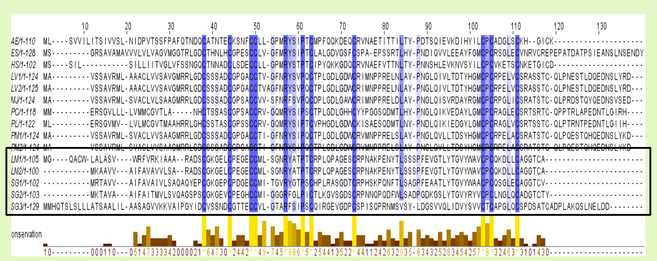
Cells survive *in vitro* over 5 days
Trypan blue (blue)
Eosin Y (Red)



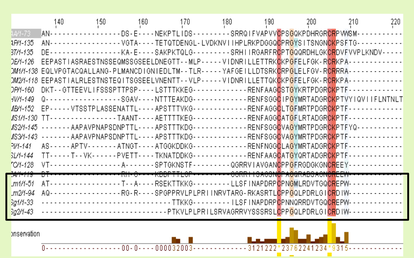
No mitosis within 5 days
DAPI (Blue)

4. HEMOCYTE ACTIVATION FACTORS

Putative hemocyte activation factors



Astakine 2-like peptides



Plasmatocyte spreading peptide (PSP)-like peptides

***In vitro* tests ongoing**

5. DISCUSSION AND PROSPECTS

- The hemocytes cultured with our conditions have a prolonged survival *in vitro* (> 5days).
- Putative cytokines, similar to crustacean astakines and insect plasmatocyte-spreading peptides, are present in the locust genome.
- The *in vitro* culture may be utilized for functional characterization of the immune-associated locust cytokines.

References
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Matsumoto H., S. Tsuzuki, A. Date-Ito, A. Ohnishi, Y. Hayakawa 2012. Characteristics common to a cytokine family spanning five orders of insects. Insect Biochem. and Biol., Vol. 42, No. 6, pp 446-457